REMARKS

Claims 1, 2, 4-6, 8, 9, 20, 45 and 46, as herein amended, and claims 12, 14, 17, 18, 23, 25, 28 and 29, as previously presented are pending in the application. Claims 3, 7, 10, 11, 19, 21, 22, 30, 33, 34, 49 and 50 have been withdrawn as being directed to a non-elected invention ,and claims 13, 15, 16, 24, 26, 27, 31, 32, 35-44, 47 and 48 have been cancelled without prejudice or disclaimer. Applicant respectfully contends that the grounds of rejection set forth in the action with regard to the cancelled claims are rendered moot.

Claims 2, 5, 6, 45 and 46 are objected-to for reciting certain claim informalities. Applicant has amended these claims to overcome the objections as follows. Claims 2 and 6 have been amended to recite "The method" rather than "A method," and to recite "the human blood plasma or serum." Claim 5 has been amended to recite "a non-cellular fraction of blood from a human group or population." Claims 45 and 46 have been amended to recite "a cancer." Applicant respectfully requests that the Office withdraw these objections in view of his amendments.

1. The claims as amended fulfill the requirements of 35 U.S.C. §112, first paragraph

Claims 2, 6, 45 and 46 stand rejected for failing to satisfy the enablement requirement of 35 U.S.C. §112, first paragraph. The Action states that "the specification, while being enabling for detecting a product amplified from total extracellular RNA from plasma or serum of a human, [it] does not reasonably provide enablement for performing the methods recited in claims 2, 6, 45 and 46 wherein the overexpression of a tumor-associated extracellular RNA species in the human blood plasma or serum can indicate that the disease is any kind of neoplastic disease . . . and the human can be determined to have any kind of cancer."

Applicant has amended claims 2 and 6 recite that the neoplastic disease is characterized by the expressed tumor-associated RNA. Applicant thus limits the scope of the claims to detecting neoplastic disease only when the RNA detected in human blood plasma or serum is a characteristic feature of said neoplastic disease. Applicant notes that the Office appears to require the claimed method to identify specific cancers. In addition to the limitations set forth in the amendments presented herein, Applicant respectfully contends that the invention would be fully enabled if the claimed methods merely identify a cancer characterized by the expressed tumor-associated RNA, if only insofar as identifying cancers with common molecular characteristics that are thus amenable to

common target-specific treatments. Applicant respectfully contends that these amendments overcome the asserted grounds of rejection, and respectfully request the Examiner withdraw these grounds of rejection.

2. The claims as amended fulfill the requirements of 35 U.S.C. §112, second paragraph.

Claims 1, 2, 4-6, 8, 9, 12, 14, 17, 18, 20, 23, 25, 28, 29, 45 and 46 stand rejected under 35 U.S.C. 112, second paragraph as being indefinite. Applicant has amended these claims to overcome the grounds of rejection as follows.

Claim 1 has been amended to clarify that the human RNA species recited in step (c) is identical to the human RNA species recited in step (b). Similarly, Applicant has amended claim 1 to clarify the relationship between amplification of the RNA species from blood plasma or serum and the reference amplified product.

Claim 2 has been amended to clarify how detection of an amplified product as recited in claim 1 can be used to indicate the existence of a neoplastic disease.

Claims 4 and 8 have been amended to clarify the identity of the amplified product in step (b) versus the reference amplified product in step (c).

Claim 5 has been amended to clarify that the RNA species recited in step (b) is the same as the human RNA species recited in step (c). Similarly, Applicant has amended claim 1 to clarify the relationship between amplification of the RNA species from blood plasma or serum and the reference amplified product.

Claim 6 has been amended to clarify how detection of an amplified product in claim 5 can be used to indicate the existence of a neoplastic disease.

Claims 9 and 20 have been amended to provide antecedent basis for the limitation to the reference range.

Claim 45 has been amended to clarify that the human in claim 9 has cancer.

Claim 46 has been amended to clarify that the human in claim 20 has cancer.

3. The claims are not rendered obvious by the cited prior art.

Claims 1, 4, 5, 8, 9, 12, 20 and 23 are rejected under 35 U.S.C. §103 as being obvious over the teachings of Balazs in view of the teachings of Korneluk.

McDONNELL BOEHNEN HULBERT & BERGHOFF LLP
300 South Wacker Drive
Chicago, Illinois 60606
(312) 913-0001

The Supreme Court has set forth the methodology for determining obviousness for patent claims. First, the scope and content of the prior art must be assessed, and then compared with the claims taken as a whole. This analysis must be performed in view of the person of ordinary skill in the art, and requires a determination of the level of skill of the person of ordinary skill. Finally, any objective evidence of non-obviousness, like commercial success or the failure of others, must be considered. *Graham v. John Deere Inc.*, 148 USPQ 459 (1966). The burden is on the Office to assert a prima facie case of obviousness, and the Office must consider all rebuttal evidence of non-obviousness provided by Applicant. In particular, with regard to the question of obviousness and the teachings of the prior art, the Office must take into account all the evidence of record, including evidence that refutes or rebuts the factual bases for the asserted *prima facie* case. *In re Sullivan*, 498 F.3d 1345 (Fed. Cir. 2007). Finally, the Office cannot use an Applicant's own disclosure, the written description of Applicant's invention, or any of the prior art, to reconstruct the invention using hindsight. *KSR v. Teleflex Int'l.*, 127 Sup. Ct. 1272 (2007).

The Office asserts that the Balazs reference teaches "extracting total extracellular RNA from blood plasma or serum from a human; amplifying or signal amplifying quantitatively or qualitatively a portion of the extracted RNA or cDNA therefrom to produce an amplified product or signal, using primers or probes specific for a human RNA species or cDNA therefrom; and detecting quantitatively or qualitatively the amplified product or signal as recited in claim 1, wherein the amplified product is produced from a tumor related RNA or cDNA produced therefrom. . .." Applicant respectfully disagrees, and contends that a correct understanding of the teachings of the Balazs reference rebuts the asserted obviousness determination.

An important but subtle distinction must be made in considering what the Balazs reference actually teaches. Specifically in contrast to the teachings of Applicant's specification, the Balazs teachings require the absolute requirement that a nuclease inhibitor (specifically, an RNase inhibitor) be mixed with whole blood prior to separating plasma from the cellular fraction of the blood. Applicant respectfully contends that the Balazs reference cannot be properly understood without considering the teaching in Balazs that a nuclease is required. This is because the consequences of following the Balazs teachings impacts whether what Balazs and Applicant teach are in fact the same, *i.e.*, whether any RNA detected using the Balazs teachings is in fact "extracellular RNA" as that term is used in Applicant's specification.

In view of what was know in the art at the time of the instant invention, it was well recognized that nucleases, specifically RNases, existed in blood plasma or serum, and it was expected that these enzymes would degrade any extracellular RNA that might otherwise be present in plasma or serum. Such RNases (ribonucleases) were expected to degrade the relatively fragile RNA within seconds. Moreover, RNases were reported to be elevated in the blood of cancer patients. It is important to note, as illustrated by references contemporaneous with the instant invention, that this was the understanding of the worker of ordinary skill in the art well after publication of the Balazs reference, i.e. this understanding was not changed even after disclosure of the Balazs reference. Specific reference to this understanding is included in the following references of record: Komeda *et al.* (Cancer, 1995, 75:2214-2219) and Pfleiderer (Int. J. Cancer, 1995, 64: 135-139).

The Komeda reference teaches that one aim of the reported study was "[t]o examine whether free mRNA in blood could also be detected" (p.2215), and further states that "[i]t was impossible to detect free RNA extracted from Hep G2 cells when they were diluted once with control blood" (p.2216), using RNA isolation and RT-PCR amplification methods substantially similar to the methods taught in the cited references. Pfleiderer, who also used RT-PCR amplification methods, teaches that the author "tested whether free . . . RNA in peripheral blood would also be detected . . .. As shown in figure 2, even high concentrations of . . . RNA (corresponding to 1% tumour cells) in peripheral blood were not detectable . . . indicated rapid and complete removal of free . . . RNA . . . by degradation . . ." (p.136). Further, the reference states that ". . . intact tumour cells are the *only* source of positive RT-PCR results. Free tumour RNA in blood, which might be released from cells . . . was not sufficiently stable to be detected . . ." (p. 137; *emphasis added*).

These references stand in contradistinction to any interpretation of the Balazs reference by the Office as teaching that extracellular RNA could be amplified or detected from blood plasma or serum prepared in the absence of an RNase inhibitor. Rather, the Balazs reference is limited to detecting specific RNA species in the plasma of cancer patients only provided that a nuclease inhibitor is present in the preparation at all times. The reference provides no teaching that any such RNA detected in a plasma sample would be *extracellular* RNA, *i.e.*, RNA present in the blood sample as extracellular RNA prior to any experimental manipulation of the sample. This is because the added nuclease inhibitor would be expected to protect from nuclease degradation any

McDONNELL BOEHNEN HULBERT & BERGHOFF LLP
300 South Wacker Drive
Chicago, Illinois 60606
(312) 913-0001

intracellular RNA released during sample preparation. This is particularly true when plasma is isolated from blood. For example, intracellular RNA could be released into the sample due to cell rupture occurring during centrifugation of the specimen.

In keeping with the understanding of the art, the Balazs reference requires the inactivation of such ribonucleases by adding an RNase inhibitor prior to isolating plasma as a condition for detecting RNA from plasma. This requirement is explicitly stated in the reference:

- Balazs (WO), abstract: "under the constant effect of a reliable RNase inhibitor…."

- Balazs (WO), pg 4: "The RNase-handled sample of this same RNA plasma was ineffective."

- Balazs (WO), pg 13: "This task is accomplished by the process as described in Claim 1. The degradation of RNA or its fragments is prevented by the use of an effective and reliable RNase inhibitor that does not induce RNA exudation from the cells, where this inhibitor is used early during specimen collection of the cellular biologic liquid. The fact that the RNase inhibitor does not induce RNA exudation from the cells prior to and during their removal is significant, because this sensitive method can identify even small amounts of contamination." (emphasis added)

- Balazs (WO), pg 14: "The following describes the invention in more detail. The cellular biological liquid (such as blood, exudates, etc.) is mixed with a reliable RNase inhibitor that does not generate RNA cell leakage as early as during specimen collection, and the cells are removed. The total RNA of the resulting acellular biological liquid is mixed with a watery medium with continuous action of the RNase inhibitor …." (emphasis added)

- Balazs (WO), pg 16, Example: " 10 ml blood with 20 IE heparin are taken and immediately mixed with a solution of RNase inhibitors such as RNasin…. The blood plasma is separated as quickly as possible." (emphasis added)

This requirement is consistent with the identified RNA being intracellular in origin, and also consistent with the teachings of Komeda and Pfleiderer. It is notable that although Balazs asserts without support that the RNAase inhibitor should not generate RNA cell leakage or induce RNA exudation from the cells, Balazs fails to provide any method to prevent cell rupture and RNA release during the separation of plasma from whole blood, such as during centrifugation. Since centrifugation is the standard method of separating plasma from blood, consistent with the teachings

of Komeda and Pfleiderer and the understanding of one skilled in the art, the skilled artisan at the time the invention was made would have attributed any RNA detected from plasma to be confounding intracellular RNA that is released during centrifugation and cell separation. Furthermore, one skilled in the art would understand Balazs to teach away from any detectable extracellular RNA existing *in vivo*, in view of the requirement that an RNase inhibitor must be present. If there would not have been any reasonable expectation that circulating extracellular RNA exists *in vivo* (i.e., in the presence of RNases known to be present therein, as evidenced by the Komeda and Pfleiderer references), then there could have been no reasonable expectation that the species could be detected following peripheral blood draw.

In contrast, Applicant's specification teaches the presence of extracellular RNA in plasma and serum, irrespective of the addition of an RNase inhibitor. The methods taught in the instant specification do not require addition of an RNase inhibitor prior to separation of plasma. This is because the Applicant recognized, as Balazs and the rest of the art did not, that adding an RNase inhibitor prior to separating the cellular and acellular fractions of blood would stabilize any *intracellular* RNA released from cells during the separation process, and thus provide contaminating intracellular RNA into the plasma sample. One of ordinary skill would recognize this deficiency in the method disclosed in the Balazs reference, and would understand that as a consequence Balazs neither teaches a method that could be used to (unambiguously) detect extracellular RNA in blood plasma, nor describes the existence of extracellular RNA in blood. The instant inventor found, surprisingly, that extracellular RNA is sufficiently stable even in the purported presence of serum RNases that it can be amplified and detected in human blood plasma or serum without adding RNase inhibitors, as evidenced by detection of extracellular RNA species using the methods disclosed in the instant specification. Adding RNases to blood prior to separating the cellular from the acellular portions thereof is thus not only unnecessary to stabilize extracellular RNA, but can stabilize any artifactually-produced *intracellular* RNA inadvertently released from blood cells during plasma sample separation. Although the Balazs reference recognizes that intracellular RNA contamination should be avoided, its own teachings subvert its intention to avoid detecting these artifactual intracellular RNA species. Balazs fails to provide any way to avoid or overcome the confounding presence of intracellular RNA, and thus provides no teaching that contradicts the understanding in the art as a whole regarding the presence of amplifiable extracellular RNA in plasma. The only

source for methods that unambiguously show the extracellular RNA can be detected from blood plasma or serum comes from Applicant's disclosure. It is established law that using Applicant's specification to produce a hindsight reconstruction of the invention from the prior art is improper for asserting a prima facie case of obviousness. *KSR v. Teleflex Int'l., Inc. Id.*

Furthermore, Applicant respectfully contends that knowledge of a method to amplify and detect *intracellular* RNA extracted from cells, as disclosed by Korneluk et al, would not have provided reasonable expectation that the same method could be applied to *extracellular* RNA from plasma or serum, since the integrity and structure of extracellular RNA in plasma could not be presumed to be sufficiently identical to intracellular RNA. Any presumption that the structure of cellular RNA is the same as RNA that endogenously circulates in plasma as extracellular RNA was without basis at the time of the invention, and is not provided by either the Balazs or the Korneluk references. The expected structural differences between extracellular (substantially or completely degraded) and intracellular (substantially intact) RNA are supported by subsequent art. RNA endogenously present in plasma has been subjected to apoptotic processes or nucleases, and thus appears to be fragmented or otherwise altered in comparison to cellular RNA. Distinction between cellular RNA and extracellular RNA has recently been noted by Zhou et al (Cancer Letters, 2008, 259:50-60), newly presented herein, stating:

- "it [the data presented] suggests that the exRNA [extracellular RNA] is different from intracellular RNA" (abstract).
- "We also explored some of the biological characteristics of the cell-free RNA. We found that expression of β-actin was *not the same* between intracellular and exRNA. Wong et al (Ann. NY Acad. Sci. 2006, 1075:174-178) … found that there was higher expression of the 5' segments compared to the 3' segments, indicating that the circulating RNA tends to be fragmented, and that the 5' ends are predominant. It is possible that the fragmentation of exRNA [extracellular RNA] may have been one of factors that led the differences in expression of β-actin between intracellular and extracellular RNA" (page 58, italics added).
- "Finally, our study indicated that there are differences in expression levels of different genes in the intracellular and exRNA (β-actin). This could be a result of a high amount of fragmentation of some exRNA sequences." (page 59, final paragraph, emphasis added)

Thus, the understanding of the skilled worker in the art several years after the Balazs reference was published contradicts the Office's position that Balazs et al. "teach extracting total extracellular RNA from blood plasma or serum". The express requirement of the Balazs teachings for invariably including an RNase inhibitor is inconsistent with extracellular RNA existing *in vivo* in

blood plasma or serum, since true extracellular RNA species would necessarily have to exist in blood plasma or serum prior to experimental manipulation and prior to the addition of RNase inhibitors. The Balazs reference can thus be seen requiring the conclusion that its methods did not and could not detect extracellular RNA, because if such RNA were stable enough to survive in blood plasma or serum in vivo it would not require the addition of RNase inhibitors, and if it were not so stable it would be degraded prior to experimental manipulation and the addition of RNase inhibitors. In fact, the only RNA whose existence in and detection from blood plasma or serum would be expected to be influenced by the experimental introduction of RNase inhibitors would be *intracellular* RNA artifactually released from damaged blood cells during isolation of the plasma or serum fractions of blood.

Thus, properly understood the Balazs reference does not teach detection of extracellular RNA in blood plasma or serum, a teaching the Action admits is expressly absent from the Korneluk reference. Thus, neither the Balazs nor the Korneluk reference would be understood by the skilled worker to suggest that extracellular RNA could be detected in blood plasma or serum, a disbelief consistent with the understanding in the art in the face of the Balazs reference as evidenced by the Komeda, Pfleiderer and Zhou references.

In addition, Applicant respectfully points out that the teachings of the Balazs reference are restricted completely to clinical cancer patients, as evidenced *inter alia* by these excerpts from the reference (as above, all citations are set forth herewith with reference to the translated text):

- "The invention concerns a method for detecting the specific mRNA sequence (target sequence) of substances of cancer cell origin…." (first sentence of spec.)

- "RNA transcripts or their specific fragments are released or deposited in detectable amounts and in a form protected against degrading enzymes only by malignant cells." (translated doc., page 7).

- "…after in vitro amplification, the malignancy test was positive only in the case of cancer, but not in the case of the precancerous adenomas." (translated doc., page 10).

- "Because of the special properties of malignant cells and tumors, the method is suitable as a malignancy test…." (translated doc., page 12)

- "Claim 1. A malignancy test (cancer test) by in vitro enzymatic amplification of the specific RNA sequence of the substances of cancer cell origin…." (translated doc., underlined for emphasis)

These citations make clear that the Balazs reference affirmatively teaches that amplifiable RNA is only present in plasma when it is from cancer cells, due to the putatively "special properties" of cancer cells. There is no teaching, and hence no anticipation, that amplifiable mammalian RNA can be detected in plasma from humans without cancer, or when the RNA is not of cancer cell origin. As such, Applicant respectfully contends that the pending claims, which make comparison to RNA from humans without disease, are not anticipated by the Balazs reference, because the reference not only does not teach mammalian RNA from plasma or serum of a human without cancer, but actually teaches against it, even stating as example that plasma RNA could *not* be detected in those with premalignancy:

> Over 50% of adenomas with precancerous changes in the large intestine have ras oncogenes activated by point mutation (particularly Ki-ras), while cancerously changed adenomas have these oncogenes in a somewhat lower percentage. The mutation appeared most frequently with the Ki-ras oncogenes in the position of codon 12, and GGT is mutated to GAT in both conditions. The malignancy tests conducted without prior amplification of said mutated oncogene sequence from blood plasma RNA gave a negative result in both conditions, while after in vitro amplification, the malignancy test was positive only in the case of cancer, but not in the case of the precancerous adenomas. This example clearly shows that the invention, in spite of its increased sensitivity, is a reliable malignancy test. (p. 10)

Applicant respectfully contends that the Balazs reference thus contradicts an interpretation that its methods could be used to detect extracellular mRNA from blood plasma or serum from a human without cancer or other disease.

The Korneluk reference does not cure these deficiencies of the Balazs reference. The Korneluk reference is limited to detecting RNA isolated from cancer cells, i.e. *intracellular* RNA, and is completely silent regarding the existence of extracellular RNA. The combination of the Balazs and Korneluk references thus do not render obvious Applicant's invention, since taken together they do not teach detection of extracellular RNA species. Furthermore, the Korneluk reference does not cure the deficiency of the Balazs reference regarding detection of extracellular RNA in the plasma of humans without cancer. Korneluk provides no teaching or suggestion that RNA can be amplified from the plasma of humans without disease. In the absence of any teaching by Balazs or Korneluk that RNA can be amplified from the plasma of humans without disease, and since Balazs specifically teaches away from detecting plasma RNA in any human without cancer,

McDONNELL BOEHNEN HULBERT & BERGHOFF LLP
300 South Wacker Drive
Chicago, Illinois 60606
(312) 913-0001

Applicant respectfully contends that it can not be considered obvious to apply a reference range based upon amounts or concentrations of extracellular RNA in the plasma from humans without disease. One skilled in the art would not have had any reasonable expectation that reference amounts or concentrations of plasma RNA from humans without disease could be defined, since one skilled in the art would have no expectation that RNA could be amplified from the plasma of humans without disease.

Applicant thus respectfully contends that he has rebutted the asserted obviousness determination made against claims 1, 4, 5, 8, 9, 12, 20 and 23, and respectfully requests that the Examiner withdraw this ground of rejection.

Claims 14 and 25 stand rejected under 35 U.S.C. §103 as being obvious over the teachings of Balazs in view of the teachings of Korneluk. For these claims, the Office concedes that the references do not teaching applying the inventive methods to humans not diagnosed with cancer, but asserts that they are nonetheless obvious because this is a mere substitution and the motivation for making such a substitution arises from the expectation that prior art elements will perform their expected function to achieve their expected results, relying on MPEP 2144.06, 2144.07 and 2144.09. Applicant respectfully traverses this ground of rejection.

Applicant respectfully contends that the evidence set forth above establishes that the existence of extracellular RNA in plasma or serum was not in the prior art due to the deficiencies of the cited references. This ground of rejection presumes that the prima facie case of obviousness asserted against the other claims stands, and thus the differences recited in these claims are not sufficient to make them nonobvious. On the contrary, Applicant respectfully contends that the contemporaneous evidence from the art establishes that the Balazs teachings would not be understood to enable the skilled worker to have any reasonable expectation of success in achieving the claimed invention. There could be no reasonable expectation of success on the part of the skilled artisan that methods known to work with intracellular RNA, as disclosed by the Korneluk reference, would be similarly applicable to extracellular RNA. It was not until the teachings of the instant specification that methods for detecting extracellular RNA in the plasma of humans without disease were provided. Applicant thus respectfully contends that this is not an instance where "prior art elements will perform their expected function to achieve their expected results," because the critical element, extracellular RNA, was not a known "prior art element," there was no "expected function"

since the status of any such RNA (substantially degraded or substantially intact) was both completely unknown and unpredictable until the instant invention, and thus there were no results to be expected. Accordingly Applicant respectfully contends that he has rebutted the asserted obviousness determination made against claims 14 and 25 and respectfully requests that the Examiner withdraw this ground of rejection.

Claims 17, 18, 28 and 29 stand rejected under 35 U.S.C. §103 as being obvious over the teachings of Balazs in view of the teachings of Korneluk. For these claims, the Office concedes that the references do not teaching populations of a specific gender or age group or who engage in behaviors like smoking that are known to increase risk of neoplastic disease. The Action asserts that they are nonetheless obvious because this is a mere substitution and the motivation for making such a substitution arises from the expectation that prior art elements will perform their expected function to achieve their expected results, relying on MPEP 2144.06, 2144.07 and 2144.09. Applicant respectfully traverses this ground of rejection.

Applicant asserts the same reasoning and comes to the same conclusions as in his argument with regard to claims 1, 4, 5, 8, 9, 12, 20 and 23 and with regard to claims 14 and 25. The deficiencies of the cited references have been set forth above, and there are thus no "expected function" since the status of any such RNA (substantially degraded or substantially intact) was both completely unknown and unpredictable until the instant invention, and thus there were no results to be expected. Furthermore, because the status of RNA in the plasma of humans without disease was completely unknown and unpredictable until the instant invention, there could have been no *expected* results. Accordingly Applicant respectfully contends that he has rebutted the asserted obviousness determination made against claims 17, 18, 28 and 29 and respectfully requests that the Examiner withdraw this ground of rejection.

Finally, the Action cites *In re Rose* for the proposition that the combination of old elements cannot be non-obvious in the absence of unexpected results. Applicant notes his argument set forth extensively above, establishing the deficiencies of the Balazs reference that preclude it from supporting the position that extracellular RNA was an "old element" known in the art. The contemporaneous references cited herein illustrate plainly that the skilled worker did not understand Balazs to teach that stable, intact extracellular RNA could be isolated and amplified from blood plasma or serum. Moreover, the skilled worker would not have understood the cited references to

render obvious detecting extracellular tumor-associated RNA in blood plasma or serum from individuals without clinical cancer, and that Applicant's detection thereof was surprising and unexpected. Applicant respectfully contends that the evidence presented herein establishes that the claimed invention is both surprising and unexpected, and that this evidence rebuts the asserted obviousness determination. Applicant thus respectfully requests that the Examiner withdraw these grounds of rejection.

CONCLUSIONS

Applicant believes that all pending claims are in condition for allowance, and respectfully request that the pending claims be passed to issue.

If Examiner Lu believes it to be helpful, he is invited to contact the undersigned representative by telephone at (312) 913-0001.

Respectfully submitted,
McDonnell Boehnen Hulbert & Berghoff LLP

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By: /Kevin E. Noonan/
Kevin Noonan, Ph.D.
Reg. No. 35,303

McDONNELL BOEHNEN HULBERT & BERGHOFF LLP
300 South Wacker Drive
Chicago, Illinois 60606
(312) 913-0001